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Levels of persistent organic pollutants in larvae of the damselfly *Ischnura elegans* (Odonata, Coenagrionidae) from different ponds in Flanders, Belgium

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A B S T R A C T

We investigated the accumulation of persistent organic pollutants in damselfly larvae (*Ischnura elegans*) in sixteen ponds in Flanders (Belgium), widely differing in the surrounding land use. Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and hexachlorobenzene (HCB) were measured. From all targeted PBDE-congeners, only three congeners (IUPAC nos. 47, 99, 100) were above the limit of quantification (LOQ). The $\sum$PBDE concentrations ranged from <LOQ up to 0.51 ng g^{-1} ww. From the targeted PCB-congeners, thirteen were detectable (IUPAC nos. 95, 99, 101, 105, 118, 138, 149, 153, 156, 170, 180, 183, and 187). A high variation in $\sum$PCB concentrations was observed between the ponds, ranging from <LOQ (0.67 ng g^{-1} ww) up to 9.91 ng g^{-1} ww in the damselflies from the pond at Sijsele. In all investigated Flemish ponds, p,p'-DDE concentrations were >LOQ (0.20 ng g^{-1} ww) with values up to 3.30 ng g^{-1} ww in the pond at Hamme. In fifteen ponds, the HCB concentrations were >LOQ (0.05 ng g^{-1} ww) with values up to 0.24 ng g^{-1} ww. For the available data in the literature a comparison with different species was done for some of the sampled ponds. The monitored ponds can be separated in three groups based on their contamination. The first group is characterised by a relative low POP content ($\sum$PBDEs, $\sum$PCBs, HCB). Group 2 contained more HCB and p,p'-DDE than the overall mean while this was the case for PBDEs and PCBs in group 3. The vectors of both contaminated groups are situated nearly perpendicular which is suggesting a different pollution sources.

1. Introduction

The ubiquitous use of plastics and textiles increased considerably since industrialisation. In order to improve their chemical and physical properties, additives which might contain persistent pollutants, are added. Waste treatment or direct leaching results in increasing concentrations of persistent pollutants in the environment. Also other persistent pollutants, such as pesticides enter the environment mainly through non-point sources such as agriculture runoff (Wang et al., 2007). Persistent Organic Pollutants (POPs) are a group of contaminants with comparable characteristics. Due to their persistence and lipophilic nature, these pollutants bio-accumulate in lipid tissue. Even in isolated alpine freshwater systems, POPs are present and accumulate in invertebrate tissues (Bizzotto et al., 2009). Due to this bio-accumulation, the concentrations of POPs are higher in biota at the top of the food chain (Gustafsson et al., 1999; Li et al., 2008; Walters et al., 2011).

The accumulation of POPs might cause various adverse effects in both, wildlife and humans, including disruption of the endocrine, reproductive, and immune systems, as well as behavioural problems, cancer, diabetes, and thyroid problems (Schwarzenbach et al., 2010). Rignell-Hydbom et al. (2004) for example, demonstrated a negative correlation between serum levels of CB-153 and p,p'-DDE with sperm mobility in humans.

Biomonitoring has proven a precise and reliable tool to monitor the presence of POPs in natural systems (e.g. Bervoets et al., 2004; 2009). Using biota to monitor POPs has several advantages. Since organisms accumulate these toxic substances during their entire lifetime, their tissue concentration is a reflection of an accumulation over a longer period and not a measure at a given moment. Additionally, not all pollutants that are present in the aquatic environment are bioavailable. Contaminants can be unavailable by irreversible binding

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at soil particles. Biomonitoring will therefore give a better insight in the availability and ecological risk of contaminated sites. Biomonitoring studies of POPs used a wide variety of taxa, including crayfish, midge larvae, mussels and fish (Bervoets et al., 2004, 2009; Covaci et al., 2005; Gewurtz et al., 2003; Schilderman et al., 1999).

The aim of this study was to investigate the accumulation of POPs in damselfly larvae (Insecta, Zygoptera). Damselfly larvae occupy an intermediate position in food webs being important predators of zooplankton and many aquatic insects including midge larvae, while being themselves eaten by fish. Their intermediate position in the trophic food chain of water bodies might give insight in biomagnification of POPs. When the surface water of a pond is contaminated, damselfly larvae can not avoid uptake by escape. Contact with pollutants or uptake through food or respiratory organs will inevitably result in accumulation (Hardersen and Wratten, 2000). Damselflies have a complex life cycle with the aquatic larval stage followed by a terrestrial flying adult stage and therefore have the capacity to link aquatic and terrestrial ecosystems, including the transfer of pollutants (Stoks and Córdoba-Aguilar, 2012). Damselflies have been used as monitors of environmental quality (Osborn, 2005) but never for specific pollutants.

As study species we chose *Ischnura elegans*, a species inhabiting different types of standing waters that are common throughout Europe (Dijkstra, 2006). In Flanders, damselflies receive specific attention in relation to nature conservation and are all protected by law. Larval concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and hexachlorobenzene (HCB) were measured. Furthermore, the accumulated levels were related to literature data of different species and environmental concentrations. As far as we are aware, this is the first study that measures POPs in field-collected damselflies.

2. Material and methods

2.1. Study ponds

In sixteen ponds, spread over Flanders (Belgium), larvae were captured for POP analysis (Table 1, Fig. 1). To have a rough estimate of the contamination, ponds with a different use of the surrounding land were selected. In all the ponds, a minimum of ten larvae with a total fresh weight of approximately 400 mg was needed to obtain enough material for POP analyses. Fourteen of the selected ponds are artificially created by peat or sand extraction, while the other two ponds have a natural origin (creek or swamp). The surface of the selected ponds ranges from 2500 m² to 1 km².

Table 1
Concentrations of organic contaminants (ng g^{-1} ww) in damselfly larvae from ponds in Flanders, Belgium.

Ponds	No. site	N[a]	Lipids (%)	∑ PCBs	∑ PBDEs	HCB	p,p'-DDE
Englegemvijver (Hombeek)	1	10	1.56	3.16	0.15	0.24	1.44
Oude Durme (Hamme)	2	48	1.88	0.95	0.05	0.13	3.30
Fort (Liezele)	3	24	2.13	2.66	0.40	0.16	1.29
Durmen (Merendree)	4	27	1.72	1.50	<0.02	0.10	0.39
Meibosvijvers (Sijsele)	5	35	2.36	9.91	0.27	0.16	1.11
Polder (Bazel)	6	38	2.60	0.71	0.03	0.11	0.72
Meer (Weerde)	7	26	0.94	8.58	0.19	0.11	0.88
Fort 8 (Hoboken)	8	37	2.16	1.00	0.12	<0.05	0.23
Warande (Dessel)	9	57	1.79	2.06	0.09	0.13	0.48
Fort (Kallo)	10	14	2.65	3.99	0.17	0.08	0.90
Polder (Hoboken)	11	25	1.88	3.90	0.51	0.13	0.50
Walenhoek (Niel)	12	37	2.12	<0.67	0.08	0.09	0.23
Hazewinkel (Willebroek)	13	49	1.34	0.80	0.18	0.11	0.40
De Maat (Dessel)	14	50	1.79	1.42	0.06	0.22	0.74
Maelesbroek (Geel)	15	46	1.75	0.78	<0.02	0.08	1.44
Het Vinne (Zoutleeuw)	16	12	1.57	<0.67	<0.02	0.11	0.94

[a] N = number of sampled larvae.

2.2. Sample collection

All larvae were captured with a macro-invertebrate dip net along a 100 m stretch of the bank. All ponds were sampled in spring 2007. To avoid an effect of age and size on pollutant concentrations, only specimen of the last larval stage (F1) was sampled. This stage can be recognised by the presence of short wing paths which do not exceed the fifth abdomen segment. Larvae from the same pond were pooled to obtain enough material need to estimate the targeted POP. Samples for the POP analysis were stored at −20 °C in polypropylene recipients.

2.3. Analyses of persistent organic pollutants

The following POPs were targeted: HCB, hexachlorocyclohexanes (α-, β-, and γ-HCH), *p,p'*-DDT and its metabolites, *p,p'*-DDE, *p,p'*-DDD, PCBs (sum of 19 congeners identified according to IUPAC numbers: 28, 52, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194, and 199), and PBDEs (congeners 28, 49, 47, 99, 100, 153, 154 and 183). Methods used for the extraction, clean-up, and analysis of POPs were previously validated (Jacobs et al., 2002) and are briefly described below.

The available amount (0.4 to 2 g) of damselfly larvae tissue was ground with anhydrous sodium sulphate and extracted with 100 mL n-hexane/acetone (3/1, *v*/*v*) in hot Soxhlet extraction mode. The lipid content was determined on an aliquot of the extract (see below), while the remaining extract was cleaned up on 8 g acid silica (40% concentrated H_2SO_4, *w*/*w*). The analytes were eluted with 25 mL hexane/dichloromethane (1/1, v/v). The eluate was concentrated to 100 μL on a rotary evaporator and a gentle nitrogen stream. The lipid content (%) of damselfly larvae was determined for each sample based on weight, by heating a known aliquot of extract at 105 °C for 1 h.

Detection and quantification of compounds were performed using a mass spectrometer (Agilent MS 5973, Palo Alto, California, USA) coupled to a gas chromatograph (Agilent GC 6890, Palo Alto, California, USA). As such, PBDEs and HCHs were analyzed with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) in ECNI (electron capture negative ionization) mode, while PCBs and DDTs were analyzed with a HT-8 capillary column (30 m × 0.22 mm × 0.25 μm) in EI (electron ionization) mode.

Recoveries of target compounds ranged between 72% and 80%. Multilevel calibration curves ($r^2 > 0.999$) were created for the tested intervals, which included the whole concentration range found in our samples. The method performance was assessed through rigorous internal quality control, which included daily check of calibration curves and regular analysis of procedural blanks and certified material CRM 349 (PCBs in cod liver oil), for which the differences between obtained and the certified values were within 15%. After blank subtraction, the limit of quantification (LOQ) was set at 3 ∗ SD of the value obtained in the procedural blanks. Method limits of quantification (LOQ) for individual PCB congeners ranged between 0.05 and 0.20 ng g^{-1} ww. For PBDE, HCB and p,p'-DDE, LOQ values were 0.02, 0.05 and 0.2 ng g^{-1} ww, respectively.

2.4. Statistical analyses

POP profiles: to assess the relationships among the different pollutants and to group similar ponds, we applied a hierarchical clustering on principle components (HCPC) (Husson et al., 2011). Basically, one first runs a principal component analyses (PCA). The resulting distance matrix serves as an input for the hierarchical clustering (HC). Finally, the optimal cluster partitioning is obtained by minimizing

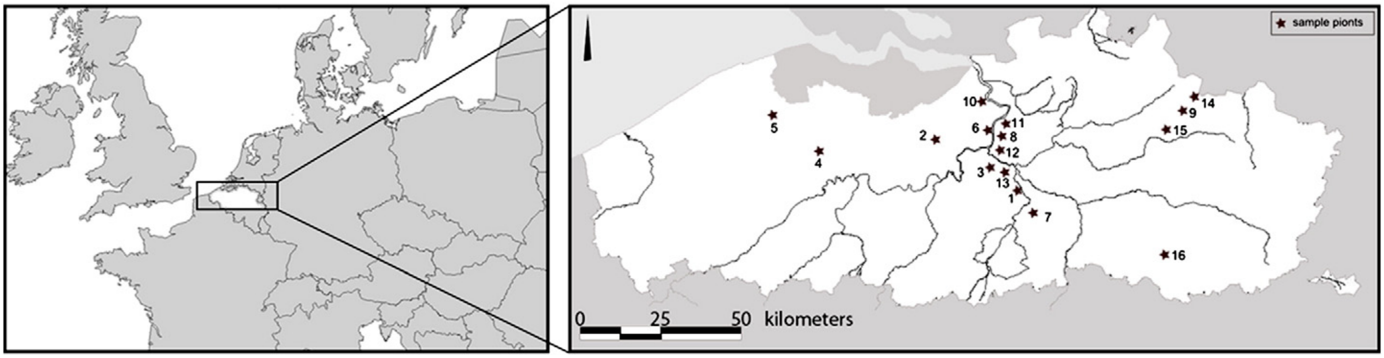

Fig. 1. Sampling locations spread over Flanders indicated with stars and corresponding numbers.

$\Delta(q)/\Delta(q+1)$ where $\Delta(q)$ = between-clusters inertia increase when moving from q-1 to q clusters. To describe the resulting groups, we applied a v-test. The v-test compares the mean of the individuals of group q with the general average. Since the v-test is based on the active variables which were used to determine the groups, the resulting p-values are only informative and only provide an indication of the “significance” of a given deviation. Data were standardised prior to analysis to attribute equal weights to each pollutant. PCA was based on the Euclidian distance. For HC, we applied both Ward's and unweighted pair-group average (UPGMA) methods.

Lipids and POPs: the impact of the POP concentrations on lipid content was assessed by fitting linear regressions with lipid content as the dependent variable. We fitted models with the individual components and the components combined. Since the concentrations of the individual components were correlated (see Results and discussion) we used the PCA-axes to assess the joint impact of POPs.

All analyses were performed in the statistical package R (version 2.13.2, R Development Core Team, 2011). For HCPC, we used the PCA and HCPC functions of the FactoMineR package (Le et al., 2008). For multiple linear regression, we used the lm function of the stats package.

3. Results and discussion

3.1. Persistent organic pollutants in damselfly larvae

The rather small numbers of captured larvae (Table 1) at half of the sites can have several reasons. First, the suitability of the environment, such as size and depth of the pond, nutrient concentrations, vegetation and fish presence determine densities and species variation (Geijskes and Tol, 1983; Nilsson, 1997). Secondly, sub-lethal contaminant concentrations can drastically affect population dynamics (Wirth et al., 2002) and the organism densities (De Jonge et al., 2008; Neamtu et al., 2009; Sugiura, 1992).

PBDEs are one of the most important brominated flame retardants (BFRs) extensively used in electronic equipment, textile, household and transport products (de Wit, 2002). From all targeted congeners, only three congeners (IUPAC nos. 47, 99, 100) were above LOQ. The $\sum$PBDE concentrations ranged from LOQ up to 0.51 ng g^{-1} wet weight (Table 1).

PCBs were globally manufactured on a large scale, with main applications in the electricity industry. Although the production of PCBs is prohibited under the Stockholm Convention on POPs, their release into the environment still occurs from the large-scale disposal of electrical equipment and secondary emissions disperse, from soil to atmosphere and from sediment to water (WHO, 2010). From the targeted PCB-congeners thirteen were detectable (IUPAC nos. 95, 99, 101, 105, 118, 138, 149, 153, 156, 170, 180, 183, and 187). A high variation in $\sum$PCB concentrations was measured, ranging from LOQ (0.67 ng g^{-1} ww) up to 9.91 ng g^{-1} ww in the damselflies from Sijsele.

p,p'-DDE is the most abundant metabolite of 1,1,1-trichloro-2,2'bis (p-chlorophenyl) ethane (p,p'-DDT). DDT was used for many decades to control malaria and pest insects worldwide. Since the 1970s, the production and use of DDT have been prohibited in North America and Europe. However, there is strong evidence that commercial mixtures with DDT are still produced and used in developing countries (Beard, 2006; Becker et al., 2010; Guo et al., 2009). The metabolite p,p'-DDE can still be found in the environment, also in Europe (Bizzotto et al., 2009; Covaci et al., 2005; Hendriks et al., 1998; Vermeulen et al., 2010; Vigano et al., 2007). In all Flemish ponds, p,p'-DDE concentrations were above LOQ (0.2 ng g^{-1} ww) with values up to 3.30 ng g^{-1} ww in the Oude Durme.

HCB was mainly produced as a fungicide for seed treatment, but was also used as a flame retardant in plastics, rubbers and paints (Pacyna et al., 2003). In fifteen ponds, the HCB concentrations were above LOQ (0.05 ng g^{-1} ww) with values up to 0.24 ng g^{-1} ww.

3.2. PCB and PBDE congener profiles

In all ponds, except Walenhoek (Niel), at least 1 of the 13 measured PCB congeners was detectable (Fig. 2). In the two ponds with high $\sum$PCB levels measured in the larvae (Meer and Meibosvijver), the concentrations of the different congeners were in the same order of magnitude. The congeners PCB-153 (28.4 ± 6.1% of $\sum$PCB) and PCB-138 (12.4 ± 4.3%) were dominating in most other ponds. The same congeners were dominant in crayfish captured in Swedish lakes and in the River Meuse in The Netherlands (Holmqvist et al.,

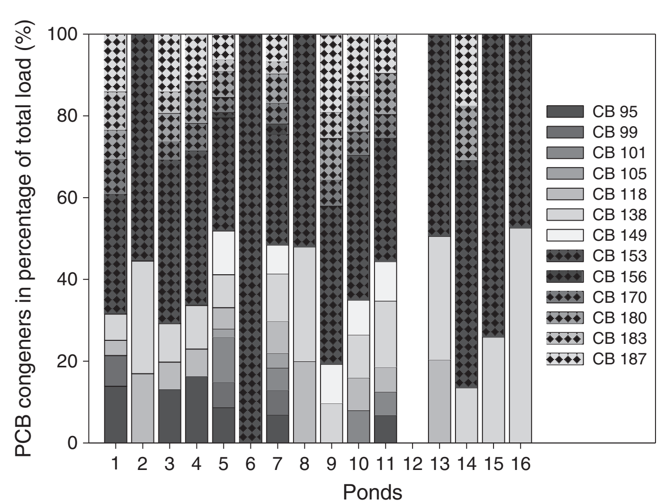

Fig. 2. Percentage profile of PCB congeners in damselfly larvae samples for each of the 16 study ponds.

2005; Schilderman et al., 1999). The dominance of heavier homologues is typical for predators (Bizzotto et al., 2009). The high portion (52.1 ± 14.56%) of heavy congeners (CB 138, 149, 153 and 156) in the total PCB load of the studied damselfly larvae corresponds with their predator behaviour (Bizzotto et al., 2009).

Only 3 (BDE− 47, 99, 100) out of 8 PBDE congeners were detectable in the damselfly larvae. BDE-47 was measured in damselfly larvae sampled from 13 of the 16 ponds with the highest concentration for Fort (Liezele) (0.30 ng g^{-1} ww). In contrast to BDE-47, BDE-99 was present in 1 pond, namely Polder (Hoboken), with 0.27 ng g^{-1} ww. Congener BDE-100 was measured in 5 ponds with a concentration up to 0.09 ng g^{-1} ww. The dominance of BDE-47 followed by BDE-99 and BDE-100 was consistent with the general patterns found in other aquatic organisms like zooplankton, snails, mussels and fish (Hu et al., 2010; Roosens et al., 2010). Debromination of higher brominated congeners often results in the congener BDE-47, which partly explains its dominance (Stapleton et al., 2004). For example, debromination of BDE-100 results in BDE-47 and debromination of BDE-153 results in BDE-99 and subsequently in BDE-47 (Gandhi et al., 2006).

3.3. Pond differences in POP profiles

The PCA analyses returned two informative PCA-axes which together explain 70.68% of the variation (PCA 1: eigenvalue = 1.58, variance = 39.53%; PCA 2: eigenvalue = 1.25, variance = 31.15%). The first axis correlated strongest with ∑PCB's ($r = 0.82$) and ∑PBDEs ($r = 0.78$). The second axis with p,p'-DDE ($r = 0.84$) and HCB ($r = 0.61$) (Fig. 3). As a consequence, ∑PBDEs and ∑PCBs explained slightly more of the variation among the ponds than HCB and p,p'-DDE. The Hierarchical clustering returned 3 groups (Fig. 3 and Table 2). The first group is characterised by a relative low POP content (∑PBDEs, ∑PCBs, HCB). Group 2 contained more HCB and p,p'-DDE than the overall mean while this was the case for PBDEs and PCBs in group 3. Both Ward's and UPGMA method gave the same results.

The ∑PCB and ∑PBDE concentrations ($r = 0.66$, $p = 0.005$) and those of HCB and p,p'-DDE ($r = 0.50$, $p = 0.05$) in the damselflies were strongly correlated. The correlation coefficients between PBDEs and PCBs were already reported before (Covaci et al., 2005; Hale et al., 2001). The vectors of both groups are situated nearly perpendicular in the PCA (Fig. 3) indicating that the distribution of these groups of pollutants is independent. Beside differences in degradation, it is reasonable to believe that this also indicates different pollution sources for the measured POPs. As reported in the literature, p,p'-DDE and HCB are expected to be widespread historically due to their general use in agriculture and pest control (Wang et al., 2009). PCB and PBDE are expected to originate from industrialized and urban areas by atmospheric deposition (Belpaire et al., 2011b; Blanchard et al., 2001; Toms et al., 2008).

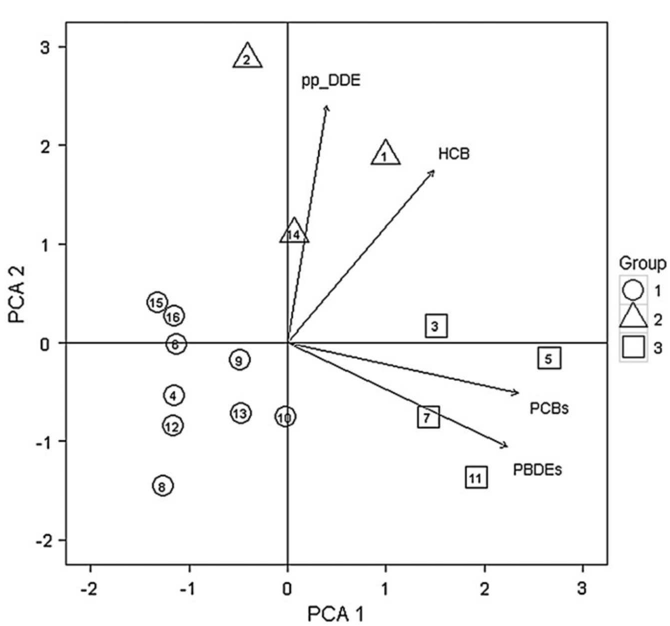

Fig. 3. PCA diagram of the measured accumulated POPs (∑ of ng g^{-1} ww). Eigenvalues (% cumulative variance); axis 1: 1.58 (39.53%), axis 2: 1.25 (70.68). The corresponding pond names and concentrations can be found in Table 1.

Table 2
POP characterisation of the 3 cluster groups (Fig. 3).

Group	POP	Mean ± SE (ng g^{-1} ww)		v-Test	p-Value
		Group	Overall		
Group 1	PBDEs	0.10 ± 0.01	0.13 ± 0.01	−2.71	0.007
	PCBs	1.35 ± 0.36	2.67 ± 0.70	−2.13	0.033
	HCB	0.08 ± 0.02	0.14 ± 0.04	−2.04	0.042
Group 2	HCB	0.20 ± 0.03	0.13 ± 0.01	2.73	0.006
	p,p'-DDE	1.83 ± 0.76	0.94 ± 0.18	2.3	0.021
Group 3	PBDEs	0.34 ± 0.07	0.14 ± 0.04	3.21	0.001
	PCBs	6.26 ± 1.76	2.67 ± 0.70	2.95	0.003

Although it was reported that lipid content, as a part of the total energy budget, can be an indicator of the degree of contamination (De Coen and Janssen, 2003), we found no relationships between the lipid content and the different POP concentrations (all $p > 0.500$).

3.4. Comparison of POP levels in aquatic biota

For some of our ponds, POP measurements in other biota, sediment and water samples were available from other studies (Belpaire et al., 2011b; Bervoets et al., 2005; Covaci et al., 2005) (Table 3). To compare the data, it was necessary to sum certain congeners (PBDE nos. 47, 99, and 100 and PCB nos. 28, 52, 101, 118, 138, 153, and 180). We found highest POP levels in larvae from Meer. This agrees with levels previously measured in sediment, zebra mussels and eel from the same ponds (Bervoets et al., 2005; Belpaire et al., 2011b).

There is a large difference in the accumulated POPs among the investigated organisms of the same pond. Damselfly larvae have the lowest accumulated concentrations of PCBs, PBDEs and HCB followed by mussel, carp and eel. A possible reason for the difference between the contamination load in damselfly larvae and the other analysed organisms is possibly the relatively short time span the larval damselflies spend in the water. *I. elegans* is bivoltine in Belgium, France and Germany (Corbet et al., 2006). In southern Europe, it is even multivoltine, while it is mainly univoltine in northern England, Italy and The Netherlands. The other target species spend several years in the water.

Another reason for this difference may be the degree of contact with the sediment. As in many other studies (Covaci et al., 2005; Guzzella et al., 2008; Li et al., 2008), the concentration of lipophilic POPs in the sediment (of the ponds where data were available) are much higher than concentrations found in the surface water. Since POPs show a high affinity for fine suspended particulate matter, the pollutants in the water phase will settle down and accumulate in the sediment (Fernandez et al., 2005). At three stations where the information was available, the accumulation of PCBs in damselfly larvae was approximately 10% of the concentration found in the sediment. Damselfly larvae are intermediate predators. The main uptake route of POPs for other invertebrate predator species was found to be the water (Bizzotto et al., 2009). Mussels also feed in the water phase but since they are filter feeders, the large amount of water which passes over their gills can explain the higher values found in these

Table 3
Concentrations of organic contaminants (ng g^{-1} wet wt) in aquatic organisms and sediments (ng g^{-1} dry wt) and water (ng L^{-1}) and lipids (%) based on current and previous studies of a subset of the study ponds.

Ponds	Lipids	∑ 7 PCB's	∑ 3 PBDE's	HCB	p,p'-DDE	Reference
Damselfly larvae						
Fort 8 (Hoboken)	2.16	0.68	0.12	<0.05	0.23	This study
Meer (Weerde)	0.94	5.07	0.19	0.11	0.87	This study
Oude Durme (Hamme)	1.88	0.64	0.05	0.13	3.30	This study
Walenhoek (Niel)	2.12	0.40	0.08	0.09	0.23	This study
Warande (Dessel)	1.79	1.23	0.07	0.13	0.48	This study
Mussels						
Fort 8 (Hoboken)	0.83	20.0	0.75[b]	<0.23	2.70	Bervoets et al. (2005); Covaci et al. (2005)
Meer (Weerde)	0.66	112	0.46[b]	0.40	8.30	Bervoets et al. (2005); Covaci et al. (2005)
Oude Durme (Hamme)	2.34	33.1	n.a.	0.28	6.60	Bervoets et al. (2005)
Walenhoek (Niel)	1.56	5.59	0.26[b]	0.24	1.30	Bervoets et al. (2005); Covaci et al. (2005)
Carp						
Oude Durme (Hamme)	2.4	52[a]	6[b]	0.28	41.00	Covaci et al. (2005)
Eel						
Meer (Weerde)	5.1	7753	n.a .	n.a.	n.a.	Belpaire et al. (2011b)
Sediment						
Fort 8 (Hoboken)		5.10	n.a.	0.40	0.05	Bervoets et al. (2009)
Meer (Weerde)		50.00	n.a.	n.a.	<0.05	www.vmm.be
Oude Durme (Hamme)		8.40	<0.05	n.a.	3.20	www.vmm.be
Water						
Meer (Weerde)		60.4	n.a.	n.a.	<0.05	www.vmm.be
Oude Durme (Hamme)		11.00	<0.05	n.a.	3.20	www.vmm.be

n.a. = not available.
[a] Summation of total measured PCBs.
[b] Summation of total measured PBDEs.

organisms (Bervoets et al., 2004). In carp and eel, the higher levels of POPs can be explained by their sediment dwelling behaviour in combination with their long life span (Belpaire et al., 2011a; Bervoets et al., 2005).

Finally, due to the lipophilic nature of the POPs, organisms with high lipid content such as eel and carp, will accumulate more than insect larvae or crustaceans (Belpaire et al., 2011b; Bizzotto et al., 2009).

4. Conclusions

Our study indicates that the accumulation capacity of damselfly larvae makes them useful for passive biomonitoring of POPs. We indicated the presence of accumulated POPs in damselfly larvae collected from ponds spread over Flanders. We observed a great variation in POP concentrations among the study ponds, which was largely consistent with published data in other organisms in these ponds. An advance of using damselfly larvae instead of the other commonly used organisms is the accurate reflection of the present bioavailability of the contamination. The relative short aquatic lifespan (bivoltine) makes the monitoring accurate and more time specific when sampling occurs in the period from egg to capture.

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